

Atty Dkt. No.: 10030468-1  
USSN: 10/686,092

## **AMENDMENTS**

### **In the Claims:**

1. **(Currently Amended)** A method of identifying a sequence of a nucleic acid that is suitable for use as a substrate surface immobilized normalization probe, said method comprising:

(a) identifying a plurality of candidate probe sequences for a target nucleic acid based on at least one selection criterion;

(b) empirically evaluating each of said candidate probe sequences under a plurality of different experimental conditions to obtain a collection of empirical data values for each of said candidate nucleic acid probe sequences for each of said plurality of different experimental conditions, and for each member of said plurality of different experimental conditions:

(i) providing an array of candidate nucleic acid probes immobilized on a surface of a solid support, wherein said array includes a substrate surface immobilized nucleic acid candidate probe for each of said identified candidate probe sequences; and

(ii) subjecting said array to said member of said plurality of different experimental conditions to produce gene expression data;

(c) evaluating said gene expression data by clustering said candidate probe sequences into one or more groups of candidate probe sequences based on each candidate probe sequence's collection of empirical gene expression data values to produce clustered probe sequences, wherein each of said one or more groups exhibits substantially the same performance across said plurality of experimental conditions; and

(d) evaluating any remaining ~~non-clustering probes~~ candidate probe sequences not among said clustered probe sequences for candidate probe sequences that satisfy a signal intensity threshold and exhibit substantially no variation in signal under said plurality of different experimental conditions to

Atty Dkt. No.: 10030468-1  
USSN: 10/686,092

identify any candidate probe sequences of said plurality that are suitable for use as a substrate surface immobilized normalization probe.

2. (Original) The method according to Claim 1, wherein said at least one selection criterion employed in said identifying step (a) is chosen from:
  - (i) proximity to the 3' end of said target nucleic acid's corresponding mRNA transcript;
  - (ii) base composition; and
  - (iii) lack of homology to other expressed sequences of said target nucleic acid's organism.
3. (Currently Amended) The method according to Claim 2, wherein all three of said selection criteria (i), (ii) and (iii) are employed is in said identifying step (a).
4. (Original) The method according to Claim 3, wherein said identifying step (a) is further characterized by employing parameters that minimize the number of identified candidate probe sequences that overlap with each other.
5. (Cancelled)
6. (Currently Amended) The method according to Claim 1, wherein each member of said plurality of different experimental conditions is a different ~~tissue/cell line~~ differential gene expression assay performed on a tissue or cell line.
7. (Original) The method according to Claim 1, said clustering step (c) comprises:
  - (i) obtaining an expression vector for each of said candidate probe sequences using said candidate sequence's collection of empirical data values;
  - (ii) deriving a similarity matrix for the set of said candidate probe sequences from said candidate probe sequences' expression vectors; and

Atty Dkt. No.: 10030468-1  
USSN: 10/686,092

(iii) grouping said candidate probe sequences based on their derived similarity.

8. (Original) The method according to Claim 7, wherein those candidate probes that have substantially similar expression patterns are grouped together.

9. (Original) The method according to Claim 1, wherein the clustering step employs an affinity threshold or another stringency controlling parameter.

10. (Currently Amended) The method according to Claim 1, wherein a candidate probe sequence ~~is considered to exhibit~~ exhibits substantially no variation in signal under said plurality of different experimental conditions if its log ratio is not significantly different than zero across said plurality of different experimental conditions.

11. (Original) The method according to Claim 10, wherein said log ratio is between about 0.5 and -0.5.

12. (Previously Presented) The method according to Claim 1, wherein said plurality of different experimental conditions is at least 2.

13. (Original) The method according to Claim 12, wherein if no non-clustering probes are present after said clustering step (c), said evaluating step (d) is not performed.

14. (Original) The method according to Claim 1, wherein at least some of said steps are carried out by a computational analysis system.

15. (Original) A computer-readable medium having recorded thereon a program that identifies a sequence of a nucleic acid that is suitable for use as a substrate surface immobilized normalization probe according to the method of Claim 1.

Atty Dkt. No.: 10030468-1  
USSN: 10/686,092

16. (Original) A computational analysis system comprising a computer-readable medium according to Claim 15.

17 - 25. (Cancelled)

26. (Currently Amended) A method of identifying a sequence of a nucleic acid that is suitable for use as a substrate surface immobilized normalization probe, said method comprising:

- (a) identifying a plurality of candidate probe sequences for a target nucleic acid based on at least one selection criterion;
- (b) empirically evaluating each of said candidate probe sequences under a plurality of different experimental conditions to obtain a collection of empirical gene expression data values for each of said candidate nucleic acid probe sequences for each of said plurality of different experimental conditions;
- (c) evaluating said gene expression data by clustering said candidate probe sequences into one or more groups of candidate probe sequences based on each candidate probe sequence's collection of empirical gene expression data values to produce clustered probe sequences, wherein each of said one or more groups exhibits substantially the same performance across said plurality of experimental conditions; and
- (d) evaluating any remaining ~~non-clustering probes~~ candidate probe sequences not among said clustered probe sequences for candidate probe sequences that satisfy a signal intensity threshold and exhibit substantially no variation in signal under said plurality of different experimental conditions to identify any candidate probe sequences of said plurality that are suitable for use as a substrate surface immobilized normalization probe; and
- (e) recording the identified candidate probe sequences on a computer-readable medium; and
- (f) reporting said identified candidate probe sequences to a user.

Atty Dkt. No.: 10030468-1  
USSN: 10/686,092

27. (Previously Presented) The method according to Claim 26, wherein said at least one selection criterion employed in said identifying step (a) is chosen from:

- (i) proximity to the 3' end of said target nucleic acid's corresponding mRNA transcript;
- (ii) base composition; and
- (iii) lack of homology to other expressed sequences of said target nucleic acid's organism.

28. (Previously Presented) The method according to Claim 26, said clustering step (c) comprises:

- (i) obtaining an expression vector for each of said candidate probe sequences using said candidate sequence's collection of empirical data values;
- (ii) deriving a similarity matrix for the set of said candidate probe sequences from said candidate probe sequences' expression vectors; and
- (iii) grouping said candidate probe sequences based on their derived similarity.

29. (Previously Presented) The method according to Claim 28, wherein those candidate probes that have substantially similar expression patterns are grouped together.

30. (Previously Presented) The method according to Claim 26, wherein at least some of said steps are carried out by a computational analysis system.